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MECHANICALLY PROCESSING COTTONSEED TO REDUCE GOSSYPOL AND AFLATOXIN LEVELS

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ABSTRACT

Cottonseed is an economical source of protein and is commonly used in balancing livestock rations; however, its use is typically limited by protein level, fat content, gossypol, and the potential for aflatoxin contamination. There are numerous studies in the literature discussing gossypol and aflatoxin toxicities in livestock and processing methods for reducing gossypol levels in cottonseed. However, there is very limited information in the literature within the last 30 years on how aflatoxin is affected by processing. Evaluation studies were conducted to determine if an extrusion process affected gossypol and aflatoxin levels in cottonseed without negatively impacting the nutritional value of the product, and if these reductions were consistent with the literature. Results from the gossypol study showed a 71 to 78% decrease in free gossypol levels due to the extrusion process, which were lower than some reported methods of processing and consistent with others. Results from the aflatoxin studies showed reductions of 50% when the

material was processed by two stages of extrusion at a temperature of 132°C. Similar reductions have been reported on roasting corn at temperatures of 140 to 143°C. The extrusion temperatures used in the evaluation studies did not significantly alter most of the nutritional values analyzed in the study. However, soluble protein was decreased at the higher temperatures. There were no significant differences in analyzed nutritional values based on multiple stages of processing. Results from the evaluation study indicate that extruding cottonseed to reduce gossypol and aflatoxin levels is an area of research that should be further explored, primarily due to the advances made in the aflatoxin and gossypol testing methods during the last 30 years.

INTRODUCTION

Cotton gins nationwide produce approximately 7.7 million metric tons of cottonseed annually, of which about 1.7 million metric tons of whole cottonseed is fed to livestock. Whole cottonseed can provide a good supply of protein, fat, and fiber. For almost all feed ingredients, there is a negative correlation between fiber and energy; however, whole cottonseed is the exception to this rule. Cottonseed meal is a co-product of the cottonseed oil extraction industry, and an estimated 1.4 million metric tons are utilized in livestock rations nationwide. Since cottonseed meal is primarily used as a protein source, the protein levels are carefully controlled during processing, yielding about 41%. Whole cottonseed and cottonseed meal have long been popular and economic sources of protein for ration formulations. However, there are limiting factors that must be considered when determining quantities of whole cottonseed and cottonseed meal in ration formulation. Significant considerations should be given to protein level and quality, fat content, gossypol, and aflatoxin.

Gossypol

Gossypol is a pigment found naturally in many gossypium species, including cotton. At least 15 gossypol related pigments or derivatives have been identified in cotton plant products (*1*). The predominately occurring pigment is polyphenolic binaphthyl aldehyde, which is yellow in color and referred to as gossypol (*2*). Gossypol is located throughout the cotton plant, with highest concentrations in the roots and significant quantities in the seed. In the seed and several other cotton tissues, gossypol is contained in small pigment glands. These glands appear as small pepper specks when a cottonseed meat is sliced open.

When these glands are intact (not ruptured), virtually all the gossypol is biologically active and is said to be in its ''free'' form. The quantity of gossypol can vary depending upon variety and environmental conditions; however, the gossypol content, of commercial varieties grown throughout the Cotton Belt, has not substantially changed in the last 40 years, even with the development of ''glandless'' varieties (*3*). Gossypol levels in gin-run whole seed are about 0.6% by weight or 6000 ppm (*4*). Relatively large amounts of gossypol may be toxic when fed to livestock, especially in its free form, these amounts vary by: duration of feeding; species; breed; age; state of rumen development; feeding level; and method of feeding (*3*).

Gossypol was first discovered by J. J. Longmore in 1886 and purified in crystalline form in 1889 by L. Marchlewski (*5*). In 1915, Withers and Carruth identified gossypol as the cause of death in pigs and calves (*5*). With this discovery, gossypol became the principle suspect whenever problems arose in feeding cottonseed or cottonseed meal to livestock. By the 1930's, it was known that swine, poultry and young ruminants were very susceptible to gossypol poisoning, and mature ruminants were very tolerant. Mature ruminants are though to be tolerant because they have acquired rumen flora that quickly metabolizes gossypol. Most ruminant nutritionists from the mid 1930's until 1980 followed Morrison (*6*) advice that ''cottonseed meal is one of the best protein supplements for dairy cows, beef cattle, and sheep''. Morrison further stated ''for calves under 3 to 4 months of age it is best not to use more than about 20% of cottonseed meal in the concentrate mixture''. In 1975, gossypol toxicity developed (in a 700 cow dairy herd in Alabama) when large amounts of cottonseed meal were fed as the single source of protein to achieve high levels of milk production, resulting in the death of 25 mature cows. Lindsey et al. (*7*) reported gossypol intoxication in mature dairy cattle consuming direct solvent extracted cottonseed meal containing high free gossypol. This research accompanied by periodic rediscoveries of gossypol poisoning in cattle (*8*; *9*; *10*; *11*; *12*; *13*; *14*) and in sheep (*15*; *16*; *17*) renewed concerns about the safety of feedstuffs containing gossypol. Collectively, these studies substantiated the remarkable ability of ruminants (post-weaning) to tolerate large amounts of gossypol for extended periods, due to rumen flora. However, it is unfortunate that major emphasis has been placed on feeding gossypol at very high levels; to demonstrate an effect, without including sufficient lower levels to define safe levels. (*2*)

Animal sensitivity to gossypol is considerably different between species and classes of animals. In general, monogastric animal and ruminants, prior to development of normal rumen function, are more susceptible to gossypol poisoning than mature ruminants (*18*; *1*). Research has defined safe levels of free gossypol in diets for monogastric animals; however, the information available on safe feeding levels for ruminant animals is limited. Therefore, the recommended safe feeding levels for ruminant animals is very conservative. Typical recommended

Table 1. Reported ''Effect'' and ''No Effect'' Levels of Free Gossypol in Non-ruminants, Based on Research Trials

	Free Gossypol Intake (ppm)		
Class of Livestock	Effect	No Effect	Reference
Yearling horses	—	115[†]	(*54*)
Weanling horses	—	348[†]	(*55*)
Young lambs	824[§]	—	(*19*)
Catfish	—	900[§]	(*56*)
Tilapia	—	1800[§Ψ]	(*57*)
Rainbow trout	1,000[†Ψ]	250[†c]	(*58*)
Shrimp (Penaeus vannamei)	—	170[§]	(*59*)

[†] Dry matter/as fed basis not reported.
[§] Dry matter basis.
[Ψ] Fed as gossypol acetic acid.

safe levels of free gossypol are presented in Tables 1 and 2, for non-ruminants, and Table 3, for ruminants. In general, limited amounts of cottonseed meal can be used in swine and poultry rations when properly managed; however, no whole cottonseed should be used. For cattle, sheep, and goats with normal rumen function cottonseed and cottonseed meal can generally be safely used when utilizing the products to meet protein requirements (i.e. ''to balance rations''), fat content may be the limiting factor when considering these products (*2*).

Binding Gossypol

Virtually all the gossypol in whole seed is in the free form (unbound). A Texas A&M survey reported that free gossypol levels in whole cottonseed ranged from 0.47 to 0.63% (4700 to 6300 ppm) (*19*). Even though all the gossypol in whole cottonseed is considered to be in the free state, analyses for free and total

Table 2. Currently Accepted Tolerance Levels for Free Gossypol in Poultry and Swine

Class of livestock	Free Gossypol Intake (ppm)	Maximum Free Gossypol Intake with Iron Salts	Reference
Broilers	100–150	400 ppm (1–2 ppm Fe: 1 ppm Free Gossypol)	(*60*)
Layers	50	150 ppm (4 ppm Fe: 1 ppm Free Gossypol)	(*60*)
Swine	100	400 ppm (1 ppm Fe: 1ppm Free Gossypol)	(*61*)

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Table 3. Recommended Safe Levels of Free Gossypol for Ruminants (*2*)

Stage of Rumen Development	Age	Free Gossypol Levels	
		ppm in Diet	Mg/lb/LW/day
Preruminant	0–3 wk	100	1.1
Transition[†]	3–8 wk	200	2.3
Functional			
Post-weaning	8–24 wk	200	3.6
Mature[§]	>24 wk	600	6.8

[†] Transition from pre-ruminant to functional ruminant begins when animals start to consume dry feed (i.e., pasture, hay, concentrate)

[§] This level is considered safe for females used for breeding. The recommended safe level for males used for breeding is 200 ppm free gossypol.

gossypol will not necessarily result in the same numbers. This is due to the use of two separate official analytical procedures for free and total gossypol. The goal in processing is to rupture the pigment glands containing gossypol, so that the gossypol binds with proteins, thus decreasing the free gossypol content. The degree of binding is also critical, since the process reduces protein quality and amino acid availability, especially with regard to lysine availability. Lysine is reported to be the primary amino acid bound to gossypol (*20*; *21*). Bound gossypol is generally considered as unavailable to the animal; however, researchers have always been concerned about bound gossypol toxicity but have not found enough evidence to include it along with free gossypol (*5*). The total gossypol content of processed cottonseed is not affected by processing; it is equal to the sum of the free plus the bound gossypol (*1*). However, during oil extraction gossypol is deposited in both the oil and the meal. The extent of binding varies with processing method; Table 4 lists the typical free gossypol levels associated with various processes.

Cotton gin by-products {CGBP} (burrs, stems, leaves, soil, etc.) present a major problem for the ginning industry. With an approximate 2.8 million tons produced annually nationwide, researchers are exploring alternative economic uses for this material. Thomasson, et al. (*22*) conducted a preliminary study to determine the feasibility of expansion processed cottonseed and CGBP mixtures as a potential valuable livestock feed. The study used an Anderson 11.4 cm (4.5-inch) Expander Cooker and focused on mixing ratios of 50:50, 75:25, 90:10, and 100:0 (% cottonseed: % CGBP). The CGBP used in this study was from Mid-South, spindle-picked, seed cotton that was ginned at the commercial size gin plant at the Cotton Ginning Research Unit, USDA/ARS, Stoneville, Mississippi. The CGBP used did not include gin motes or lint cleaner waste. The cotton-

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Table 4. Processing Methods and Their Respective Free Gossypol Levels

Processing Method	% Free Gossypol
Hydraulic[†]	0.04–0.10
Screw press[†]	0.02–0.05
Prepress solvent[†]	0.02–0.07
Direct solvent[†]	0.10–0.50
Expander solvent[§]	0.06–0.21

[†] (*1*)
[§] (*19*)

seed and CGBP were mixed using a ribbon mixer. The expander cooker was operated in the same manner as that used for mechanically cooking oilseeds. Samples for total gossypol, free gossypol, nutritional, and palatability analyses were collected pre- and post-processing. Results from Thomasson, et al. (*22*) showed that a simple, relatively low-cost expansion process could be used to produce a livestock feed from cottonseed and CGBP and reduced free gossypol levels (about 90%) in the final product.

Aflatoxin

Aflatoxin in cottonseed may limit its use. Aflatoxins, secondary metabolites of the fungus *Aspergillus flavus* and *A. parasiticus*, are acute toxins to most animals and are the most hepatoxic and hepatocarcinogenic natural agents known (*23*). *Aspergillus flavus* can propagate in any substratum capable of supporting fungal growth, especially in warm humid environments (*24*). These fungi can infect crops before and after harvest and produce aflatoxins, thereby contaminating foods and feeds (*25*; *26*). Because the production of aflatoxin is so dependent upon environmental conditions, the amount actually produced varies widely from sample to sample and year to year.

The first report of aflatoxin toxicity appeared in 1961, when contaminated peanut meal was linked to the deaths of over 120,000 turkeys and other poultry (*27*). Keyl and Booth (*28*) conducted the first comprehensive study on the effects of feeding aflatoxin to livestock and poultry. This work established the levels of aflatoxin required to produce recognizable growth effects in swine, beef and dairy cattle, and broilers and laying hens. However, the methodology available then could not detect low part per billion levels; therefore, the data on transmission of aflatoxin residues in meat were not as definite. More recent studies have established that residues of aflatoxin can be found in animal tissue (*29*; *30*; *31*; *32*;

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33; *34*). These studies suggest that low levels of aflatoxin-contaminated feed (400 ppb for cattle and swine and 150 ppb from turkeys) will result in detectable aflatoxin residues in the liver, kidney, and muscle. Theses studies further suggest that aflatoxin is eliminated from the animal tissue in a relatively short time (4 days for swine, 14 days for turkeys, and 21 days for cattle); and chickens can handle large doses (2000 ppb) with little effects (with no aflatoxin detected in the tissue after 2 days).

Mycotoxins are regulated by the Food and Drug Administration (FDA). The Federal Grain Inspection Service (FGIS) has a Memorandum of Understanding to report any over-tolerance results it finds to the FDA. In 1969, the FDA set an action level for aflatoxins at 20 ppb for all foods, including animal feeds, based on the agency's aim of limiting aflatoxin exposure to the lowest possible level. Due to animal feeding studies in the 1970's and 1980's, the agency revised its action level in 1982 to 300 ppb for aflatoxins in cottonseed meal intended for used as a feed ingredient for beef cattle, swine, and poultry (regardless of age or breeding status). The action level for cottonseed meal and other feed ingredients intended for dairy animals, animal species or uses not previously specified, or when the intended use is unknown remain at 20 ppb.

The health impacts of aflatoxin are much less precise than regulatory limits or guidelines suggest. There are no clear-cut safe levels, since the levels vary with each individual animal. The generally recommended level of aflatoxins in feed is 0 ppb. However, aflatoxin-contaminated feed can be tolerated by some animals, particularly mature ones. In general, ruminants are able to tolerate higher levels of aflatoxins and longer periods of low-level intake than simple-stomached animals. In order of susceptibility, ducklings are first followed in order by turkeys, pigs, calves, mature cattle, and sheep (*23*). The response of ruminants to aflatoxin-contaminated feed depends upon the level of toxin present, age, and species. Young, rapidly growing ruminants are more susceptible than are mature ruminants. Ingestion of aflatoxins at levels lower than FDA action levels may cause some undesirable side effects, and is dependent on such factors as age, sex and general health of the animals. Obviously, the higher the level of contamination, the greater the risk associated with feeding this material to animals.

Aflatoxin Variability

Aflatoxin detection test results are inherently variable. This can be attributed to sampling, subsampling, and analytical variability. Whitaker et al. (*35*; *36*; *37*) indicated that sampling variability, especially for small sample sizes, is the largest source of error in determining aflatoxin concentration. Sampling error is large because aflatoxin is found only in a small percentage (less than 0.1%) of the kernels in the lot (*38*). In addition, of the 0.1% a single seed concentration

may be extremely high. Because of the extreme range in aflatoxin concentrations among individual seeds in a contaminated lot, the variation among replicated samples is large. About 90% of the error associated with detection tests is due to sampling. Once the sample has been taken from the lot, the sample must be prepared for aflatoxin extraction. The entire sample must be thoroughly mixed before the subsample is collected (*39*). The subsampling variance is not as large as the sampling variance due to the large number of mixed particles in the subsample. Next, the aflatoxin is extracted by official methods (*40*; *41*). These methods involve several steps such as solvent extraction, centrifugations, drying, dilutions, and quantification, which can result in considerable variation among replicated analyses on the same subsample extract. Analytical variability generally accounts for only a small portion of the total error.

The only way to achieve a more precise estimate of the true lot concentration is to reduce the total variation associated with the tests. Sampling error can be greatly reduced by collecting a more representative sample from the lot. This can be accomplished by taking 10 or more random samples from the lot, the larger the number of samples the better. Subsampling error can be reduced by thoroughly mixing the sample prior to collecting the subsample and increasing the sample size. Replicating the tests will reduce the analytical variability.

Aflatoxin Reductions Due to Processing

There are several proposed methods of processing cottonseed to reduce aflatoxin levels. Ammoniation is a relatively common process used in Arizona and California. However, the process is not an FDA-approved practice, so the ammoniated material must be used on-farm or sold for use within the state. With proper treatment, the process has been shown to reduce aflatoxin concentrations by 95% or more (*42*). Blending is another alternative that has been used to reduce moderate concentrations of aflatoxin. The FDA does not permit the blending of contaminated and uncontaminated commodities, but does allow the mixing of different levels of contaminated commodities. Irrespective of the processing method, the final product must be retested and fall within the regulatory limits and be properly labeled. The literature on ammoniation and blending is quite clear on the effectiveness of the processes, but there are several conflicting reports on the reduction of aflatoxin levels due to cooking, extruding, or in general terms, processes that utilize relatively high temperatures and pressures. For example, Fischbach and Campbell (*43*) reported that it was necessary to raise the temperature to 300°C or higher to decompose aflatoxins and even then reductions are limited. Goldblatt (*44*) stated that a temperature of 100°C decreased aflatoxin content. A recent report by Kenkel and Anderson (*45*) suggests that roasting

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temperatures of 143 to 149°C can reduce aflatoxin levels by 40 to 50%, in corn. Very limited recent information is available in the literature on the effects of temperature and pressure on aflatoxin. However, there is information in the literature on how these parameters affect other mycotoxin levels. Katta et al. (*46*) suggests that an extrusion process with temperatures in the range of 160 to 200°C and screw speeds ranging from 120 to 160 rpm will reduce Fumonisin B_1 by 46 to 76%. Castelo et al. (*47*) reports that greater Fumonisin B_1 reductions were obtained at an extrusion temperature of 120°C as compared to 140°C. Ryu et al. (*48*) focused on Zearalenone and reported greater reductions at an extrusion temperature of 120°C or 140°C as compared to 160°C. They further suggested that reductions of 77 to 83% with mixing screws and 73 to 77% without mixing screws were obtained at an extrusion temperature of 120°C. Since the mid 1980's, much of the research on aflatoxins has focused on the development of novel biocontrol strategies and/or the development of elite crop lines ''immune'' to aflatoxin producing fungi.

The study of Thomasson et al. (*22*) has been evaluated by the Cotton Ginning Research Unit, USDA/ARS, Stoneville, Mississippi by conducting a similar study. This study expands the previous work by focusing on aflatoxin as well as gossypol reductions due to mechanical processing. This study used an extruder as the mechanical means of processing the mixtures as compared to previous work that used an expander cooker. Although the mechanical designs of the two processes are different, they produce similar products and heat the product during processing. The study was comprised of four sections: gossypol; preliminary aflatoxin; effects of extruder temperature on aflatoxin levels; and the effects of multiple pass extrusion on aflatoxin levels.

EVALUATION STUDIES

The gossypol study was an evaluation of work by Thomasson, et al. (*22*) and is the basis for utilizing mixtures of CGBP and cottonseed in lieu of strictly cottonseed. In the gossypol study, the extrusion process was evaluated in terms of gossypol reduction and nutritional value. A preliminary study was conducted to determine if the extrusion process affected the aflatoxin levels in the contaminated cottonseed, a justification for completing the remaining studies. The third study focused on aflatoxin reductions due to extruder temperature. Variations in nutritional values were also considered. The final study evaluated the effects of multiple pass extrusion on the changes in aflatoxin levels and nutritional values. The process of extruding the material multiple times was a simplified means of testing the effects of increased dwell time. The alternative to this method was reconfiguring the extruder for each of the respective dwell times of interest.

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Extrusion Equipment

The commercial-size extruding machinery at the Insta-Pro International Research and Development Facility in Des Moines, Iowa was used for this study. The machinery consisted of an Insta-Pro Model 2500 dry-extruder followed by an Insta-Pro air type belt drier to cool the material. This extruder is a single screw adiabatic extruder that generates heat through friction. It is commonly referred to as a high temperature, short-time extruder, which can achieve temperatures up to 180°C in less than 20 seconds. The inside diameter of the barrel is 16.5 cm and the overall length is 107 cm, with a constant diameter screw. The barrel was configured with two compression chambers for the purposes of this study. A schematic of the extruder barrel is shown in Figure 1. The pitch of the worm flights determines compression. Shear is determined by the size of the steamlocks, screw flight, and the adjustment of the nose bullet and cone in the last chamber of the barrel. The barrel wall and steamlocks are grooved to enhance mixing and shearing of the product being extruded (*49*).

The material was fed into the extruder through a electronic controlled volumetric feeder equipped with an agitator, which provided a relatively uniform and free-flowing material. Once the material entered the inlet chamber, it was forced into the first steamlock by the screw. Grooves in the steamlock walls allowed for a gradual build-up in pressure as the material passed through the compression chambers. When the material reached the last chamber containing the nose bullet and cone, an estimated maximum pressure of 2,750 kPa was achieved.

Extrusion is a process that applies pressure and shear to the material being extruded. In addition, the material is being internally mixed, to create a more-uniform final material. The mixing process, along with pressure and shear, produce frictional forces between the material particles and between the particles and the internal barrel components thereby heating the product being extruded. These characteristics of extrusion are dependent on one another. Therefore, these characteristics will be lumped together and defined as the extrusion process, in terms of extruder temperature.

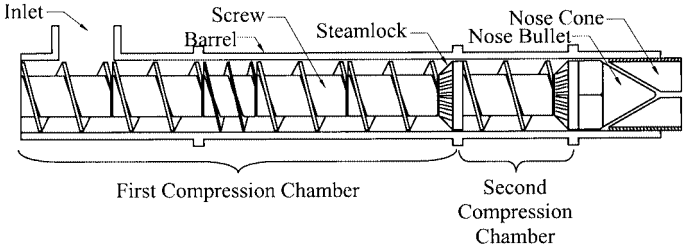

Figure 1. Extruder barrel cross-section.

Sampling and Lot Preparation

Due to the amount of manual material handling, time, and resources required for these replicated tests, relatively small lot sizes were essential. A key for determining lot sizes was the required amount of sample needed for the various analyses. The sample sizes required for gossypol and nutritional analyses were relatively small compared to the amount of sample required for aflatoxin analyses, due to the large variability and other aspects associated with aflatoxin analyses. Therefore, the sample sizes were based on the quantity of sample required for aflatoxin analyses. The sampling procedures for the aflatoxin studies were generally based on the FDA Office of Regulatory Affairs Inspectional References: Investigations Operations Manual's guide for mycotoxin sample size, which are used to obtain representative aflatoxin analysis for truckloads or other large quantities of material. A sample size of 1 to 1.5 kilograms (the FDA's operation manual suggests an individual sample size of 0.5 kg when the material has initially tested positive for aflatoxin) was selected for these studies because the material used for these tests had initially tested positive for high levels of aflatoxin; the entire amount of contaminated material used was mixed before and during lot preparation; 15 to 20 random subsamples were combined for each sample; the tests were replicated; and analyses were confirmed by a second laboratory. Lot sizes for the gossypol, preliminary aflatoxin, secondary extruder temperature, and multiple pass extrusion tests were 90, 90, 70, and 140 kilograms, respectively.

Samples for the gossypol, aflatoxin, and nutritional analyses were collected in essentially the same manner. However, the number of samples varied by test and analysis and is discussed later in the respective sections. After processing the material in the gossypol, preliminary aflatoxin, and extruder temperature tests, the entire extruded lot was placed on a large flat surface and the material was spread out uniformly before subsamples were collected. During the multiple pass extrusion tests, the extruded material was collected in several plastic tubs where subsamples were collected throughout the extruded material, before the material was reprocessed. A minimum of 15 random subsamples were collected and mixed together to produce one sample. Throughout the remaining sections of this paper, the word ''sample'' refers to a collection of subsamples, which were collected as previously discussed.

Gossypol

The gossypol study required about 850 kilograms of CGBP and 600 kilograms of cottonseed. The CGBP (not including motes from the upper moting system of a gin stand or lint cleaner waste) and cottonseed were collected during

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the ginning of typical spindle-picked Mid-South seed cotton. Burdette Gin Company in Burdette, Mississippi supplied the CGBP and the cottonseed was collected at the Cotton Ginning Research Unit, USDA/ARS, in Stoneville, Mississippi.

Various mixing ratios of CGBP and cottonseed were used in this study. Thomasson et al. (*22*) suggested that mixing ratios with less than 25% cottonseed produced a loose and fluffy product, unacceptable for its intended use as a livestock feed. Therefore, a mixing ratio of 25% cottonseed and 75% CGBP was used as a base level. The mixing ratios, in terms of percent cottonseed to percent CGBP, were 25:75, 30:70, 40:60, 50:50, and 60:40.

The test consisted of three replications, requiring a total of 15 test lots. The study was conducted as a completely randomized statistical design. A target extrusion temperature of 132°C was used. Since the lots differ in composition, water had to be added during processing to maintain the target temperature. This is due to the cottonseed having relatively high oil content in comparison to the CGBP. The oil acts as a lubricant, allowing the material to more easily pass through the barrel of the extruder.

During the mixing process, ten cottonseed and five CGBP samples were randomly collected. Nutritional analysis was performed on five of the cottonseed and five of the CGBP samples, and gossypol analyses were performed on the five remaining cottonseed samples. When the test procedures were originally developed, samples for gossypol analyses were to be collected from the CGBP bulk material and from each of the prepared lots. However, these samples were omitted in the final set of procedures, based on Dr. Calhoun's (personal communication, 1999) recommendation that using the official AOCS methods for gossypol analyses on these samples would produce highly variable and erroneous results, due to the non-homogeneity of the material. Prior to processing each lot, one mixed sample was collected for nutritional analysis. After processing, one sample was collected for gossypol analysis and one sample was collected for nutritional analysis. Dr. Calhoun at the Agricultural Research and Extension Center (San Anglo, Texas) preformed the gossypol analyses and Dairy One (Ithaca, New York) preformed the nutritional analyses.

Two methods were utilized in the gossypol analyses. The American Oil Chemist Society's standard methods were utilized in determining the total and free gossypol level, while a high performance liquid chromatographic (HPLC) procedure was used to determine the isomer percentages. The official method for free gossypol is based on extraction with an acetone-water mixture (70:30), reaction with aniline and measurement of the gossypol-aniline reaction product in a spectrophotometer at 440 nm (*50*). The official method for total gossypol is based on reacting free and bound gossypol in a sample with 3-amino-1-propanol in dimethyl formamide solution to form a gossypol-di(amino-propanol) complex. This reacted with aniline to form the gossypol-aniline reaction product that is

measured the same manner as free gossypol (*51*). These procedures measure gossypol, gossypol analogs, and gossypol derivatives having an available aldehyde function. Although not specific for gossypol, they appear to be satisfactory for use with cottonseed and cottonseed meal. However, the procedure for free gossypol has been found unsatisfactory when applied to mixed feeds, over-predicting the actual levels found in the material. Extraction with aqueous acetone results in incomplete recovery of free gossypol from feed mixtures and removes other feed constituents, which interfere in the subsequent spectrophotometric determination (*52*). High performance liquid chromatographic (HPLC) procedures have been developed which are more specific for gossypol (*53*). However, this is not an official method for determining free and total gossypol and was not used in this work or work by Tomasson et al. (*22*).

Preliminary Aflatoxin

The preliminary aflatoxin section of this study required about 900 kilograms of aflatoxin-contaminated cottonseed. The Anderson Clayton Corporation (Stanfield, Arizona) supplied the cottonseed. The cotton variety and production location were not identified. The seed was drawn from a certified pile that tested positive for aflatoxin contamination, >1,005 ppb. While preparing the material for transport, grab samples were collected and analyzed for aflatoxin content. The reported (uncertified) level was 1,650 ppb.

This study focused on the extrusion process at six processing temperatures. The temperatures were 104, 116, 127, 138, 149, and 160°C. Only a single replication of the temperatures was performed, since the primary purpose was to determine the feasibility of conducting further tests (i.e. is extrusion a possible means of reducing aflatoxin levels) and to determine the temperature levels that should be associated with further testing. Prior to creating the lots, the shipping bags were cut open so the seed would fall on the floor. Shovels were used to mix the seed. The lots were created by scooping seed randomly from the pile. The study was conducted as a completely randomized statistical design. During the extrusion process, the remaining contaminated seed was used before and between lots to adjust the extruder temperature to the proper level. During lot preparation and after each lot was processed, three random samples were collected for aflatoxin analyses. Dairy One performed these analyses.

The aflatoxin analyses for all aflatoxin studies utilized the following procedure prior to using a Veratox testing kit (Neogen Corp.): the entire sample was ground fine enough to pass through a No. 20 sieve and thoroughly mixed. The Veratox assay for aflatoxin is a competitive direct enzyme-linked immunosorbent assay that allows the user to obtain exact concentrations in parts per billion. Free toxin, in the sample is allowed to compete with an enzyme-labeled toxin (conju-

gate) for the antibody binding sites. After a wash step, substrate is added that reacts with the bound enzyme conjugate to produce a blue color. The test is read in a microwell reader to yield optical densities. The detection range for the kit is 5 to 50 ppb; therefore, if the contamination level is above 50 ppb sample dilution is required.

Extrusion Temperature

The extrusion temperature study required 850 kilograms of contaminated cottonseed. The Chickasha Cotton Oil Company (Casa Grande, Arizona) supplied roughly 1,850 kilograms of aflatoxin-contaminated cottonseed for use in this study and the multiple pass extrusion study. The cottonseed was produced in Maricopa County, Arizona, and it initially tested positive for aflatoxin at >650 ppb.

Based on the information obtained from the preliminary aflatoxin test, this study focused on extrusion temperatures of 104, 132, and 160°C. Four replications were performed for a total of twelve lots. The test was conducted as a completely randomized statistical design. Contaminated seed from the bulk supply, not used to generate the lots, was used before and between lots to adjust the extruder temperature to the proper level.

After processing, 5 random samples were collected. Four of these samples were used for aflatoxin analyses and the remaining sample was used for nutritional analysis. All nutritional and three of the aflatoxin samples collected after extrusion were analyzed by Dairy One. Neogen Corp. (Lansing, Michigan) analyzed the remaining samples.

Multiple Pass Extrusion

The multiple pass extrusion study required about 400 kilograms of aflatoxin-contaminated cottonseed drawn from the same bulk supply received from the Chickasha Cotton Oil Company. During these tests, each lot was extruded four times with samples for nutritional and aflatoxin analyses collected before each pass and after the final pass. Three replications were completed, requiring a total of three lots. The statistical design of the test was a completely randomized block design, blocked by replication. The target extrusion temperature for this study was 132°C. During processing, each lot was extruded, the material was collected in plastic tubs, samples were randomly collected, the material was reprocessed by the extruder, and the process was repeated until the lot was processed four times.

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After each pass, 5 random samples were collected: four for aflatoxin analyses and one for nutritional analysis. All nutritional and three aflatoxin samples from each processing stage were analyzed by Dairy One. Neogen Corp. analyzed the remaining samples.

EVALUATION RESULTS

Gossypol

During the mixing ratio study, an internal extruder temperature range of 130 to 135°C was maintained, and approximately 95 to 100 amperes were used to operate the extruder. The water injection rates were 38, 38, 30, 11, and 8 liters per hour for the 75, 70, 60, 50, and 40 percent CGBP mixtures, respectively. The production rates were 500, 568, 646, 750, and 791 kg per hour for the 75, 70, 60, 50, and 40 percent CGBP mixtures, respectively. As expected, the water injection rates decreased and the production rates increased as the percent of cottonseed in the mixture increased.

Gossypol results are reported in an as fed basis, as shown in Table 5. Total and free gossypol levels for the non-extruded cottonseed (0.682 and 0.693%, respectively) are consistent with values previously reported. Differences between the free and total values are attributed to analytical methods. Other studies have documented similar differences when using the two official methods that are theoretically the same. Further, there were significant differences in the free and total gossypol levels for the various mixing ratios and the levels generally decrease with the percent cottonseed in the mixture. This was expected because cottonseed contains more gossypol than the CGBP. The mean square error for the total gossypol test was 0.0007, resulting in an F-value of 153. The mean square error for the free gossypol test was 0.0001, resulting in an F-value of 2177.

Theoretically, the reported values minus the percent of CGBP in the mixture times the gossypol levels associated with extruded CGBP and this quantity divided by the percent of cottonseed in the mixture should equal the gossypol levels associated with the extruded cottonseed. When performing these calculations for total gossypol, values from 0.82 to 0.59% were obtained for mixtures containing 25 to 60% cottonseed. Theoretically, these values should be statistically equivalent to those obtained for the 100% non-extruded cottonseed. However, several significant differences were detected. Based on this information and the fact that adjusted total gossypol levels for the 25 and 30% cottonseed extruded mixtures were significantly higher than the 100% non-extruded cottonseed, it was determined that the gossypol analyses, using the official Association of Official Analytical Chemists methods, overestimated the actual gossypol levels present

Table 5. Percent Free and Total Gossypol and Isomer Ratios for Cottonseed and Cotton Gin By-products, Based on Mixing Ratios

	AOCS[Φ] Gossypol, %		Isomer % of Total[Ψ]	
Product Composition	Free[†]	Total[§]	(+)	(−)
100% Cottonseed Non-extruded	0.693a	0.682a	59.3a	40.7a
25% Cottonseed Extruded	0.060b	0.205e	57.9b	42.1b
30% Cottonseed Extruded	0.065bc	0.244de	58.4b	41.6b
40% Cottonseed Extruded	0.066bc	0.280cd	58.1b	41.9b
50% Cottonseed Extruded	0.056c	0.297c	58.2b	41.8b
60% Cottonseed Extruded	0.078b	0.356b	58.2b	41.8b

[†] Free gossypol determined by AOCS official method Ba 7–58.
[§] Total gossypol determined by AOCS official method Ba 8–78.
[Ψ] (+) and (−) gossypol isomers were determined by HPLC using 2-amino-propanol as a complexing reagent.
[Φ] AOCS–American Oil Chemist's Society.
Means in a column not having a letter in common are significantly different from the other means in the column at $\alpha = 0.05$ according to the Waller-Duncan's multiple range test.

in the material. These overestimates were expected. A more in-depth discussion of the gossypol over-estimations can be found in (*53*).

Reductions in the free gossypol levels due to the extrusion process ranged from 71 to 78% for mixing ratios of 25 to 60% cottonseed. These reductions are most likely underestimated, since the overestimations associated with the free and total gossypol levels could not be determined. Significant differences in the plus and minus gossypol isomer percentages were detected between the non-extruded and extruded mixtures. The differences were relatively small, less than a 1.4 difference between all the values. The mean square error and degrees of freedom associated with the isomer tests were 0.26 and 13, respectively.

Nutritional values for the extruded mixtures of CGBP and cottonseed are shown in Table 6. There are significant differences in several of the nutritional components, which were expected due to the varying amount of CGBP. Crude protein, net energy of maintenance, net energy of gain and total digestible nutrients significantly increased, and ash content decreased as the percent of cotton-

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Table 6. Nutritional Values of Non-extruded Cottonseed and Cotton Gin By-products and Various Mixing Ratios of Extruded Cotton Gin By-products and Cottonseed[†]

	Non-extruded		Extruded				
Nutrient Value	100% CS	100% CGBP	25% CS	30% CS	40% CS	50% CS	60% CS
Crude Protein (%)	30.0a	15.5d	16.6cd	17.4cd	17.7cbd	19.1bc	20.1b
Adjustable Crude Protein (%)	30.0a	15.5d	16.6cd	17.4cd	17.7cbd	19.1bc	20.1b
Soluble Protein (%)	21.7a	19.0a	14.8a	16.5a	9.8a	23.0a	14.8a
Acid Detergent Fiber (%)	30.3b	46.5a	47.3a	47.1a	44.5a	46.7a	43.8a
Neutral Detergent Fiber (%)	41.5c	51.8b	56.0ab	58.1a	54.5ab	55.1ab	53.0ab
Total Digestible Nutrients (%)	80.0a	37.3f	41.5de	40.3e	42.8cd	44.5bc	45.7b
Net Energy of Maintenance (Mcal/kg)	2.09a	0.81f	0.92de	0.88e	0.95cd	0.99bc	1.01b
Net Energy of Gain (Mcal/kg)	1.54a	0.04e	0.18d	0.13de	0.22cd	0.26bc	0.31b
Calcium (%)	0.19e	2.11a	1.92ab	1.90ab	1.69bc	1.39cd	1.10d
Phosphorus (%)	0.84a	0.26e	0.41d	0.42d	0.49c	0.56b	0.61b
Magnesium (%)	0.40a	0.34b	0.35b	0.36ab	0.38ab	0.38ab	0.37ab
Potassium (%)	1.22c	1.61a	1.64a	1.63a	1.53ab	1.57ab	1.48b
Sodium (%)	0.004e	0.049ab	0.051a	0.047ab	0.042bc	0.035c	0.026d
Iron (ppm)	86e	1043a	554bc	631b	581b	400d	428dc
Zinc (ppm)	41a	30cd	29d	33bc	35b	33bc	35b
Copper (ppm)	5.67a	4.00b	4.33b	4.50b	5.00ab	4.50b	4.83ab
Manganese (ppm)	14e	83a	59bc	63b	59bc	48cd	44d
Molybdenum (ppm)	1.20b	1.73a	1.25b	1.47ab	1.50ab	1.42b	1.35b
Sulfur (%)	0.29c	0.39a	0.37ab	0.37ab	0.35ab	0.32bc	0.32bc
Ash (%)	—	—	12.1ab	12.5a	11.3b	9.1c	8.6c

[†] All values based on dry matter.

Means in a row not having a letter in common are significantly different from the other means in the row at $\alpha = 0.05$ according to the Waller-Duncan's multiple range test.

— Not reported

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seed increased. Acid and neutral detergent fiber significantly increased with the addition of CGBP, but did not significantly vary with changing mixing ratios. Calcium, potassium, sodium, and iron significantly increased with increased CGBP content, while phosphorus and zinc significantly increased with increased cottonseed content. In general terms, the nutritional value of the product increased as the percent of cottonseed increased in the mixture.

Preliminary Aflatoxin

During the preliminary aflatoxin binding study, extrusion temperatures were maintained within ±1°C of the target values. The water injection rates increased from 15 to 30 liters per hour and the amperage increased from about 65 to 90 amps as the temperature increased from 116 to 160°C. In order to increase the operating temperature, the extruder nose cone had to be adjusted (i.e., resulting in an increase in pressure and shear forces within the extruder). Therefore, increasing the temperature required an increase in water injection rates, to keep the material flowing through the extruder barrel.

Significant differences in aflatoxin levels were found between the non-extruded cottonseed and cottonseed extruded at 149 and 160°C; however, there were no significant differences between the 6 temperatures used. Means and 95% confidence intervals are shown in Figure 2. The sample variability was relatively high for the non-extruded material and extrusion temperatures of 104, 127, and

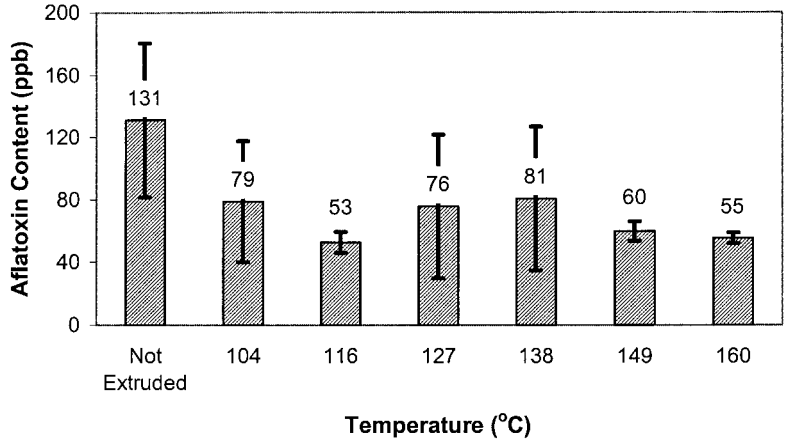

Figure 2. Aflatoxin means (columns) and 95% confidence intervals (bars) for the preliminary aflatoxin test, conducted at various extruder temperatures.

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138°C, indicating that additional replications were needed. Standard deviations ranged from 44 ppb for the non-extruded to 3 ppb for the material extruded at a temperature of 160°C. The F- and p-values for the test were 2.26 and 0.1, respectively. Although initial aflatoxin levels, as determined by the supplier, were 1,005 ppb, the maximum level detected for non-processed cottonseed was 162 ppb and the maximum level detected in the extruded cottonseed was 123 ppb.

Based on the results of this study, it was determined that additional tests would be required to determine if the extrusion process reduced aflatoxin levels in cottonseed. Additional tests should focus on fewer extruder temperatures, but maintain the range of 104 to 160°C. Further, additional tests should include more observations per treatment and more test replications.

Extrusion Temperature

During the extrusion temperature study, temperatures were maintained within ±1°C of the target values. The water injection rates were 15, 19, and 30 liters per hour for treatment temperatures of 104, 132, and 160°C, respectively. The extruder pulled an average current of 70, 80, and 86 amps for temperatures of 104, 132, and 160°C, respectively.

The statistical analysis of the aflatoxin measurements was based on a completely randomized design blocked by testing laboratory. Based on initial data plots and residual analysis, the statistical model was developed using the natural logarithm of the aflatoxin measurements. From an intuitive perspective, it was expected that if the extruder temperature affected the aflatoxin measurements, then the defining model would be based on an exponential decay of the aflatoxin levels with increased temperature and not a linear model.

A statistical analysis was conducted to determine if the aflatoxin measurements made by the Neogen Corp. confirmed the measurements made by Dairy One (i.e. testing block effects). The non-homogeneity (interaction between the laboratory and temperature) between the measurements made at the two laboratories was not significant (F-value 0.00; p-value 0.9808). Therefore, this analysis indicated that the measurements made by Neogen Corp. confirmed the measurements made by Dairy One.

Further statistical analyses were conducted using a model of the natural logarithm of the aflatoxin measurements as a function of temperature, where the interactions due to laboratory and temperature were treated as random errors. The mean square error for the analysis was 0.1655 with 46 degrees of freedom. Means and 95% confidence intervals for temperatures of 104, 132, 160°C were 182 ± 44, 137 ± 34, and 117 ± 21 ppb, respectively. Based on the analysis, there was a significant reduction (0.05 level) in aflatoxin levels as the temperature increased (F-value 7.81; p-value 0.0075). The least square means fit, means, and

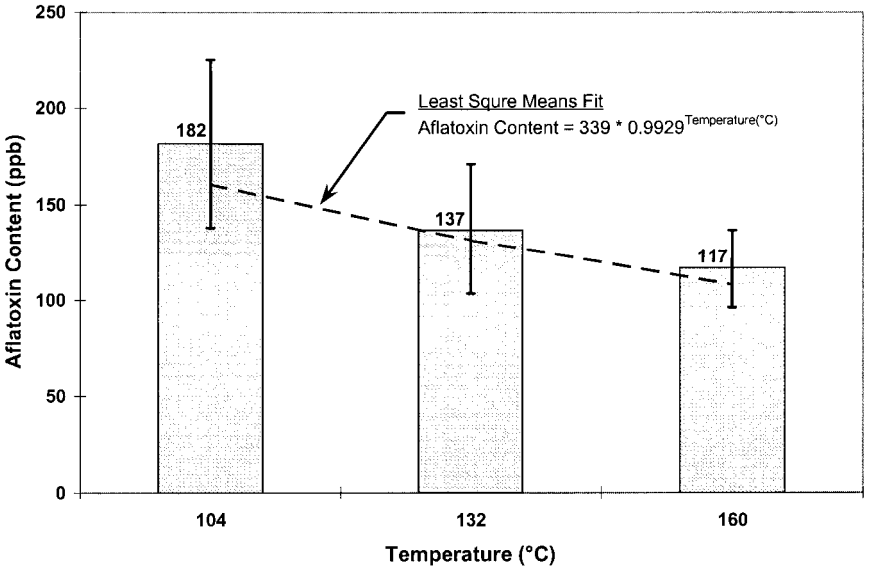

Figure 3. Aflatoxin means (columns), 95% confidence intervals (bars), and least means square fit (line) for the aflatoxin study, conducted at various extruder temperatures.

95% confidence intervals are shown in Figure 3. The slope associated with the least square means fit indicates that the aflatoxin levels are sequentially reduced by about 20% for each 20°C increase in temperature.

There were no significant differences in the majority of the nutritional values. However, there were a few notable differences. Crude protein, fiber, total digestible nutrient, net energies, and most of the mineral values exhibited relatively low variation with temperature treatments, as indicated by the corresponding p-values shown in Table 7. Soluble protein and copper were the exceptions. Soluble protein levels associated with an extrusion temperature of 104°C were significantly higher than those at 132 and 160°C. However, no significant differences were detected between the 132 and 160°C treatments. Copper values were significantly decreased as the extrusion temperature increased from 104 to 160°C. There were no significant differences in iron content of the extruded material; however, the iron value for the 104°C treatment was relatively high (702 ppm) in comparison to the other treatments (148 and 179 ppm). Two of the iron values for the 104°C treatment were 1,600 and 989 ppm, which was substantially higher than any other samples. These high values may be explained by the fact that small rocks were found in the cottonseed. Rocks tend to ''hang'' in the barrel of the extruder, which can cause damage to the extruder by marring the inside of the barrel or the screw thereby potentially depositing iron into the extruded

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Table 7. Nutritional Values for Cottonseed Extruded at Various Temperatures[†]

	Temperature (°C)			
Nutrient Value	104	132	160	p-value[ψ]
Crude Protein (%)	22.5a	22.3a	22.3a	0.9937
Adjustable Crude Protein (%)	22.5a	22.3a	22.3a	0.9937
Soluble Protein (%)	18.0a	10.8b	11.0b	0.0005
Acid Detergent Fiber (%)	43.5a	41.0a	47.0a	0.2203
Neutral Detergent Fiber (%)	58.0a	56.3a	59.6a	0.6061
Total Digestible Nutrients (%)	78.8a	77.5a	79.5a	0.4981
Net Energy of Lactation (MCAL/LB)	0.93a	0.92a	0.94a	0.5224
Net Energy of Maintenance (MCAL/LB)	0.95a	0.93a	0.96a	0.5354
Net Energy of Gain (MCAL/LB)	0.65a	0.63a	0.65a	0.5176
Calcium (%)	0.15a	0.15a	0.15a	0.8563
Phosphorus (%)	0.57a	0.58a	0.56a	0.9257
Magnesium (%)	0.32a	0.32a	0.31a	0.9079
Potassium (%)	1.26a	1.28a	1.30a	0.7266
Sodium (%)	0.009a	0.010a	0.011a	0.6964
Iron (ppm)	702a	148a	179a	0.1689
Zinc (ppm)	33a	33a	34a	0.9703
Copper (ppm)	6.0a	5.3ab	4.5b	0.0288
Manganese (ppm)	23a	19a	19a	0.3402
Molybdenum (ppm)	1.68a	1.95a	1.88a	0.8894
Sulfur (%)	0.22a	0.22a	0.21a	0.3473

[†] All values based on dry matter.
[§] Means in a row not having a letter in common are significantly different from the other means in the row at $\alpha = 0.05$ according to the Waller-Duncan's multiple range tests.
[ψ] p-values are base on F-test with 11 degrees of freedom.

material. In general, extruding the material at 132°C increased the nutritional value of the cottonseed, due to the decreased soluble protein levels.

Multiple Pass Extrusion

During the multiple pass extrusion tests, extrusion temperatures were maintained within the range of 132 to 138°C. The water injection rates were constant at 19 liters per hour and the extruder current draw ranged from 72 to 78 amperes for all stages of processing. Numerous nose cone adjustments were required to maintain a relatively constant and uniform flow rate for all the stages of processing. This is due to the changes in the physical makeup of the material, which was altered by each stage of processing.

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The statistical analysis of the aflatoxin measurements was based on a completely randomized design blocked by replication and testing laboratory. Based on initial data plots and residual analysis, the statistical model was developed using the natural logarithm of the aflatoxin measurements. From an intuitive perspective, it was expected that if increased processing (increased passes) affected the aflatoxin measurements, then the defining model would be based on an exponential decay of the aflatoxin levels with increased processing and not a linear model.

A statistical analysis was conducted to determine if the aflatoxin measurements made by the Neogen Corp. confirmed the measurements made by Dairy One (i.e. testing block effects due to laboratory). The non-homogeneity (interaction between the laboratory and number of passes) between the measurements made at the two laboratories was not significant (F-value 0.64; p-value 0.4373). Therefore, this analysis indicated that the measurements made by Neogen Corp. confirmed the measurements made by Dairy One.

Further statistical analyses were conducted using a model of the natural logarithm of the aflatoxin measurements as a function of the number of passes, where the interactions due to laboratory, replication, and number of passes were treated as random errors. The mean square error for the analysis was 0.1390. Means and 95% confidence intervals for 1, 2, 3, and 4 passes were 288 ± 84,

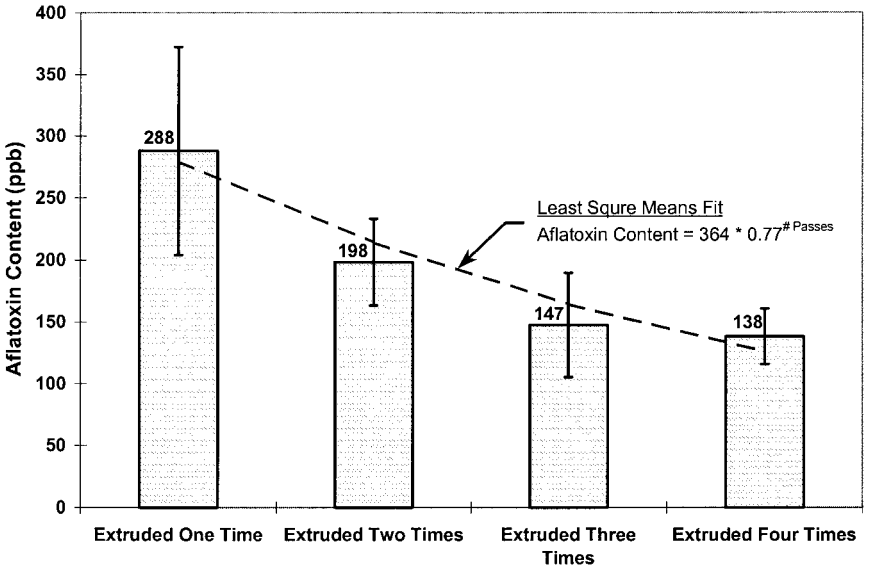

Figure 4. Aflatoxin means (columns), 95% confidence intervals (bars), and least square means fit (line) for the multiple pass aflatoxin study.

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198 ± 35, 147 ± 42, and 138 ± 22 ppb, respectively. Based on the analysis, there was a significant reduction (0.05 level) in aflatoxin levels as the number of passes increased (F-value 12.69; p-value 0.0063). The least square means fit, means, and 95% confidence intervals are shown in Figure 4. The slope associated with the least square means fit indicates that the aflatoxin levels are sequentially reduced by about 23% for each additional stage of processing. To reiterate, extruding the material multiple times was a simplified means of testing the effects of material dwell time. Results from this test should not imply that extruding the material multiple times is economically feasible; however, the tests do indicate that the extruder configuration will impact the amount of aflatoxin reduction obtained from the process.

There were no significant differences in most of the nutrient values with respect to increased processing. Phosphorus, zinc, and sulfur were the exception. These values were significantly decreased when comparing one stage of pro-

Table 8. Nutritional Values for Cottonseed Extruded Multiple Times[†]

	Number of Time Extruded				
Nutrient Value	1	2	3	4	p-value[ψ]
Crude Protein (%)	20.7a	17.4a	16.9a	16.5a	0.3867
Adjusted Crude Protein (%)	20.7a	17.4a	16.9a	16.5a	0.3867
Soluble Protein (%)	11.3a	10.3a	13.3a	9.3a	0.2557
Acid Detergent Fiber (%)	49.3a	48.8a	52.2a	50.8a	0.8124
Neutral Detergent Fiber (%)	60.8a	65.8a	65.2a	67.1a	0.5924
Total Digestible Nutrients (%)	78.3a	78.0a	77.3a	78.0a	0.8417
Net Energy of Lactation (MCAL/LB)	0.93a	0.93a	0.93a	0.92a	0.8272
Net Energy of Maintenance (MCAL/LB)	0.94a	0.93a	0.92a	0.93a	0.5909
Net Energy of Gain (MCAL/LB)	0.64a	0.63a	0.62a	0.62a	0.3691
Calcium (%)	0.15a	0.14a	0.13a	0.11a	0.1899
Phosphorus (%)	0.54a	0.46ab	0.41ab	0.35b	0.1476
Magnesium (%)	0.31a	0.28a	0.26a	0.22a	0.1746
Potassium (%)	1.29a	1.28a	1.20a	1.00a	0.2269
Sodium (%)	0.014a	0.011a	0.010a	0.007a	0.2247
Iron (ppm)	190a	206a	187a	389a	0.4265
Zinc (ppm)	33.3a	28.7ab	26.0ab	21.0b	0.0925
Copper (ppm)	4.3a	5.0a	4.0a	2.3a	0.3327
Manganese (ppm)	20.7a	20.3a	19.0a	17.3a	0.5745
Molybdenum (ppm)	2.03a	2.00a	1.63a	1.63a	0.5672
Sulfur (%)	0.20a	0.17ab	0.15b	0.16b	0.1013

[†] All values based on dry matter.

[§] Means in a row not having a letter in common are significantly different from the other means in the row at $\alpha = 0.05$ according to the Waller-Duncan's multiple range tests.

[ψ] p-values are base on F-test with 11 degrees of freedom.

cessing to four stages of processing. Although not significant, the remaining mineral values and crude protein values appeared to decrease, while the fiber values appeared to increase with increased processing, as shown in Table 8. In general, increasing the number of stages of processing, while maintaining an extrusion temperature of 132°C, did not significantly impact the nutritional value of the extruded cottonseed.

CONCLUSIONS

Reductions in the free gossypol levels due to the extrusion process ranged from 71 to 78% for mixing ratios of 25 to 60 percent cottonseed for the evaluation study, below the 90% reduction reported by Thomasson et al. (*22*). Using the mean total gossypol level obtained in the evaluation study, 0.682%, and these reduction percentages, the expected free gossypol levels would range from 0.12 to 0.20%. This range of free gossypol levels is above the hydraulic and screw press estimates previously reported by Berardi and Goldblatt (*1*). However, this range does fall between the expander solvent range reported by Calhoun et al. (*19*). The reductions from the evaluation study are most likely underestimated, since the official methods of determining free and total gossypol overestimate the levels in mixed feeds. The nutritional value associated with the extruded mixtures of cottonseed and CGBP, increased as the percent of cottonseed increased in the mixture.

The preliminary aflatoxin study indicated that the extrusion process reduced aflatoxin levels and warranted further study. The extrusion temperature test showed that aflatoxin levels were sequentially reduced by about 20% for each 20°C increase in temperature. Further, multiple pass extrusion tests indicated that aflatoxin levels were sequentially reduced by about 23% for each additional stage of processing. The results from the extrusion temperature study, showing that aflatoxin levels are affected by temperature, are consistent with work reported by Goldblatt (*44*). However, the results are inconsistent with work reported by Fischbach and Campbell (*43*), which implied that only limited aflatoxin reductions would occur if the toxin were subjected to temperatures in excess of 300°C.

Combining the least square means fits obtained from the evaluation studies, reductions of 55% (3 stages of processing), 50% (2 stages of processing), and 47% (1 stage of processing) occurred at processing temperatures of 104, 132, and 160, respectively. This information is consistent with work reported by Kenkel and Anderson (*45*), which suggested that roasting temperatures of 143 to 149°C reduced aflatoxin levels by 40 to 50% in corn. If the extreme conditions (4 stages of processing at 160°C) of the evaluation studies are applied the to combined temperature and processing equation, the resulting aflatoxin reduction

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would be 76%. This estimated reduction is well below the 95% reduction due to ammoniation reported by Gardner et al. (*42*).

The nutritional values associated with the extrusion temperature tests were not significantly changed by the increased temperatures, with the exception of soluble protein. Soluble protein was reduced as the extrusion temperature was increased. Nutritional values associated with the multiple pass extrusion study were not significantly changed by increasing the number of processing stages, with the exception of phosphorus, zinc, and sulfur. These values generally decreased with increased processing.

Based on the results of this study, further research should be conducted to determine the optimum extruder parameters required to achieve the largest reductions in gossypol and aflatoxin levels, with regards to economic feasibility.

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DISCLAIMER

Mention of a trade name, propriety product or specific equipment does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply approval of a product to the exclusion of others that may be suitable.

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